DEVELOPMENT AND MAINTENANCE OF AN IMPROVED LABORATORY COLONY OF CORN EARWORMS

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DEVELOPMENT AND MAINTENANCE OF AN IMPROVED LABORATORY COLONY OF CORN EARWORMS

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ABSTRACT

This study was conducted to establish a laboratory colony of vigorous corn earworms, *Heliothis zea* (Boddie), capable of withstanding prolonged laboratory maintenance and with the ability to retain their physiological and behavioral integrity. In a previously established colony, the insects were low in vigor (as determined by oviposition, mating, and egg hatch), possibly the result of inbreeding and heavy infestation of *Nosema heliothidis* (Lutz and Splendor). A colony was produced by crossing light-trap-collected males with disease-free laboratory females and by selecting genetic lines having high mating, oviposition, and hatch and few deformed pupae or adults. The colony was maintained for more than 70 generations by using a crossing system that minimized inbreeding. The results of this study indicated that this colony outperformed the original colony in nearly every respect.

INTRODUCTION

Reliable and reproducible research results with insects have always been hindered by the use of varied and dissimilar lines or strains of insects. Thus, the establishment of a colony of insects, reasonably uniform in certain critical parameters, is a prerequisite to any investigation. If the research result is to be extrapolated to the field, certain aspects of the insect's physiology and behavior must be similar to those of native insects. The colony must be capable of prolonged maintenance in the laboratory and, at the same time, retain its physiological and behavioral integrity. Therefore, efforts were begun 6 years ago to establish a laboratory colony of the corn earworm, *Heliothis zea* (Boddie), which would be high in vigor and reasonably uniform in certain parameters.

The original colony of insects (as represented by the baseline in table 1) was low in vigor (as determined by oviposition, mating, and egg hatch) but mated readily under laboratory conditions, a characteristic not observed in wild insects brought into the laboratory. When these parameters were measured in a sample of 225 pairs before the production of a colony free of *Nosema heliothidis* (Lutz and Splendor), oviposition and hatch were low.[3] Fifty-one percent of the females mated an average of 0.68 times per female (or 1.32 times per mated female) with a range of zero to 3 spermatophores. Females oviposited zero to 1,082 eggs per female and averaged 420. Egg hatch was zero to 61 percent and averaged 16 percent. No apparent relationship existed among the number of matings, number of eggs

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[3] Hamm, J. J., Burton, R. L., Young, J. R., and Daniel, R. T. 1971. Elimination of *Nosema heliothidis* from a laboratory colony of the corn earworm. Ann. Entomol. Soc. Am. 64: 624–627.

TABLE 1.—*Measurement results for five generations during selection and for later generations in an improved corn earworm colony*

Generation[1]	No. pairs sampled[2]	Percent mated ♀	Mean No. spermatophores per mated ♀	Oviposition per mated ♀			Percent hatch per mated ♀		
				Average	Minimum	Maximum	Average	Minimum	Maximum
G_1	19 total	80	2.0	1,285	3	2,633	44	0	67
G_1	9 retained	100	2.3	1,580	893	2,026	55	44	64
G_2	95 total	84	1.2	1,121	0	3,014	57	17	98
G_2	34 retained	100	1.3	1,560	889	3,014	87	45	98
G_3	116 total	91	2.7	1,441	0	3,483	55	0	99
G_3	9 retained	100	2.6	2,968	2,568	3,483	89	73	99
G_4	73 total	18	2.8	814	0	2,739	28	0	92
G_4	32 retained	100	2.9	1,054	96	2,739	50	7	92
G_5	199 total	77	1.5	813	0	0	15	0	0
G_5	179 retained	77	2.1	872	0	0	16	0	0
G_7	49 total	65	2.0	1,066	1	2,335	62	0	98
G_{32}	420 total	94	2.1	758	0	0	0	0	0
G_{34}	240 total	92	2.5	800	0	0	60	0	0
G_{59}	192 total	96	2.3	663	0	0	67	0	0
Baseline[3]	225 total	51	1.3	420	0	1,082	16	0	61

[1] G_1, parent generation. G_4, generation was exposed to excessive temperature (40° C) during larval-pupal stage. G_5, this and subsequent generations were caged 5 pairs per cage.
[2] Total, all pairs evaluated. Retained, pairs from which progeny were retained.
[3] Obtained from the controls of experiment conducted before this study.

oviposited, and the percentage of hatch. Inbreeding and a heavy infestation of *Nosema* may have caused the poor performance. Thus, it was desirable to introduce native genetic material in order to increase vigor.

PROCEDURES AND RESULTS

Since it was believed that males were less likely to transmit disease to progeny,[4] the colony was produced by crossing light-trap-collected males with disease-free females from the laboratory colony. Genetic lines were selected or discarded on the basis of mating, oviposition, egg hatch, and, in some cases, on the basis of growth and development of the progeny. A predetermined standard of performance was not used for all generations.

In the initial cross, in which laboratory females were crossed with wild males (generation 1 or G_1), 15 of 19 pairs mated (table 1). Progeny were retained from 9 of the pairs in which each female oviposited a minimum of 1,000 eggs free of *Nosema,* with no less than 50 percent hatch. Five unmated pairs and five mated pairs that produced less than 1,000 eggs and less than 50 percent egg hatch were eliminated. Data for pairs that were discarded were included to show the variations among pairs. *Nosema* was not found in samples of progeny from these crosses.

All possible crosses (at least two pairs per cross when available) were made among G_2. Of the 95 pairs caged, progeny were retained from 34 pairs, each of which produced at least 556 eggs with a minimum of 90 percent hatch or 2,000 eggs with a minimum of 50 percent hatch. Of the 95 pairs, 16 percent did not mate, 5 percent mated but did not oviposit, 29 percent produced fewer than 500 eggs, and 13 percent had less than 50 percent egg hatch.

Progeny from G_2, producing at least 1,000 eggs and 80 percent egg hatch, were crossed in all possible combinations in G_3. Each cross was replicated two to four times, depending on the availability of moths. Of 116 pairs caged, progeny were retained from 9 pairs, each of which produced at least 3,200 eggs with a minimum of 73 percent hatch. Of the 116 pairs, 9 percent did not mate, 13 percent mated but produced fewer than 100 eggs, 10 percent produced 100 to 500 eggs, 5 percent produced 500 to 1,000 eggs, 14 percent produced 1,000 to 1,500 eggs, 17 percent produced 1,500 to 2,000 eggs, 11 percent produced 2,000 to 2,400 eggs and, 15 percent produced more than 2,400 eggs but had less than 73

[4] Hamm et al. cited in footnote 3.

TABLE 2.—*Sample crosses for minimizing further inbreeding in a corn earworm colony*[1]

Parent line	1st generation, 1st cross	2d generation, 2d cross	3d generation, 3d cross
A	A × B and B × A = 1.	1 × 3 and 3 × 1 or (AB) × (CD) = A_1.	$A_1 \times B_1$ = 1.
B	B × C and C × B = 2.	2 × 4 and 4 × 2 or (BC) × (DE) = B_1.	B × C = 2.
C	C × D and D × C = 3.	3 × 5 and 5 × 3 or (CD) × (EA) = C.	C × D = 3.
D	D × E and E × D = 4.	4 × 1 and 1 × 4 or (DE) × (AB) = D.	D × E = 4.
E	E × A and A × E = 5.	5 × 2 and 2 × 5 or (EA) × (BC) = E.	E × A = 5.

[1] Line designations were used for convenience in making crosses and not to imply that parent lines were maintained.

percent hatch. Hatch varied from zero to 99 percent (average 55 percent) among the mated pairs discarded.

In G_4, larval as well as adult performance was evaluated. Two hundred newly hatched larvae per pair were isolated.[5] Data were obtained on the number of dead larvae, on the normal, deformed and dead pupae, and on the normal, deformed, and dead adults from each pair. Unfortunately, the larvae and pupae were exposed to excessive heat (above 40° C) for about 12 hours, which resulted in high mortality. Data from these observations were used in selecting lines for G_4.

Seventy-three pairs of G_4 moths (which represented 10 lines) were set up. Crosses were made between males and females of the same line to determine if high oviposition and hatch could be sustained with inbreeding. Only 18 percent of the pairs caged in this generation mated. Oviposition was reduced to an average of 814 per mated female with 28 percent egg hatch. The drastic reduction in mating and egg hatch was due, at least in part, to the exposure to excessive heat during the larval and pupal stages.[6] The pairs from which progeny were retained averaged 1,054 eggs with 53 percent hatch. Development was observed, and lines exhibiting excessively slow development or high rates of abnormal pupae or adults were eliminated.

In G_5, males and females of the same line were again paired (five pairs per cage). Seventy-seven percent of the pairs mated and oviposition averaged 813 with 15 percent egg hatch. Lines from which progeny were retained averaged 872 eggs with 16 percent hatch. The larvae were observed, and lines showing excessive abnormalities were eliminated. This action left five lines (A–E) which were used in a system of crossing to minimize further inbreeding in the colony (table 2). Sample crosses for three such generations are given in table 2.

This crossing system was used for more than 70 generations in maintaining the colony. The stock colony for each day consisted of an equal number of cages with six pairs of moths per cage (for both reciprocal crosses). Eggs from the cages of reciprocals were pooled for production of the new line.

Generation 6 moths were evaluated to determine oviposition, mating, and egg hatch without selection of any specific characters. Among the lines, mating ranged from 50 to 76 percent, oviposition from 504 to 1,410, and hatch from 53 to 77 percent. These results showed lower means for mating, oviposition, and egg hatch than those obtained in G_3, but showed marked increases over those obtained in G_4 and G_5.

In G_8, egg production among lines ranged from 765 to 855 per female. No correction was made for females that did not mate.

[5] Burton, R. L. 1969. Mass rearing the corn earworm in the laboratory. U.S. Dep. Agric., Agric. Res. Serv. [Rep.] ARS 33–134, 8 pp.

[6] Jackson, R. D., Daugherty, D. M., and Davidson, J. L. 1968. Effect of heat on *Heliothis zea* pupae. Am. Entomol. Soc. North Cent. Branch Proc. 23: 36. Fye, R. E. and Poole, H. K. 1971. Effect of high temperatures on fecundity and fertility of six lepidopterous pests of cotton in Arizona. U.S. Dep. Agric. Prod. Res. Rep. No. 131, 8 pp.

The colony was assessed again, after being in the laboratory for 32 generations, by sampling 420 pairs of each cross. Results indicated that mating increased over that for G_6; i.e., 93 to 95 percent of the females mated an average of 2.1 times per mated female. Average oviposition was 758 eggs per female, approximately the same as that for G_8. Hatch was not evaluated during this generation. This colony required approximately 28 days to complete a generation.

Perkins et al.,[7] in a separate evaluation, did a study of the performance of this colony on four types of rearing media. They used 240 pairs for the evaluation of each diet and showed that insects from the 34th generation, when reared on the cornmeal-soy flour-milk (CSM) diet, averaged 800 eggs per female with a 60.08-percent hatch and 2.51 spermatophores per mated female. This was near the values obtained for the 8th and 32d generations.

The colony was further evaluated for performance on three types of media after 59 generations.[8] A sample of 192 pairs (reared on a modified CSM media) had 96 percent mated females with 2.26 spermatophores per mated female, resulting in a 67-percent egg hatch from an average of 663 eggs per female.

[7] Perkins, W. D., Jones, R. L., Sparks, A. N., Wiseman, B. R., Snow, J. W., and McMillian, W. W. 1973. Artificial diets for mass rearing the corn earworm (*Heliothis zea*). U.S. Dep. Agric. Prod. Res. Rep. No. 154, 7 pp.

[8] Jones, R. L., Perkins, W. D., and Sparks, A. N. 1975. An evaluation of two new diets for the corn earworm. Ann. Entomol. Soc. Am. J. Econ. Entomol. 68(3): 349–350.

CONCLUSIONS

During its development, the new colony outperformed the old colony (as represented by the baseline in table 1) in all respects, except in percentage of mated females in G_4 and in percentage of hatch in G_5. Generations 4 and 5 were the first and second generations in which lines were inbred. Unfortunately, G_4 larvae and pupae were exposed to excessive temperature; thus, it is impossible to say how much of the reduction in mating and egg hatch was due to inbreeding and how much was due to excessive temperature. The excessive temperature during G_4 might have had a substerile effect that resulted in a low hatch in G_5, though the percentage of pairs mated increased in G_5.

After the crossing system was initiated in G_6 to minimize inbreeding, the colony performed considerably above the baseline in all parameters measured. However, the average oviposition was not as high as it was during the development of the colony or before inbreeding and exposure to excessive heat occurred.

Results of this study indicated that the laboratory colony of corn earworms was improved by mating wild males with laboratory females and by selecting those lines having high mating, oviposition, and hatch and few deformed pupae or adults. Such a colony was maintained in excess of 70 generations by using a crossing system that minimized inbreeding.

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